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AUTOMATED LIFE DETECTION DEVICES

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As man's perspective of the Universe has expanded, his own position within it has been shrinking. We may now be at the threshold of another step in this anthropomorphic degradation. That this possibility has not prevented man from pursuing the search speaks well, I think, for the species. This search for extraterrestrial life has an obvious and compelling fascination for humanity. And if it is successful, the discovery will have an immeasurable impact on man and on the course of his future.

It is necessary to define the scope and the method of carrying out such an investigation. Within the definition of scope some insight into what life is, must be sought. I shall sidestep this requirment by stating that we begin by assuming the universality of carbon-based chemistry in an aqueous environment as essential for a biota. Until and unless definitive evidence to the contrary is obtained, we will have no other significant alternative. This is only one of the several limitations now imposed on the quest for life.

The planet Mars is slated to be the first extraterrestrial body to be sampled for the existence of life because it most nearly approaches the conditions found on the Earth. Launches can only be made when Mars and Earth are in opposition. Weight and energy restrictions require further that automated devices be light and rugged, be able to withstand high

temperature of sterilization (135° C for 26 hours) and the low temperatures and pressures of a space flight, plus the shock and vibrations of take-off and landing. The instrumentation and telemetry power must be of the order of a few watts. The device must be reliable, stable and be capable of very great sensitivity in order to detect the broadest spectrum of living organisms in the smallest numbers. Finally, it must be compatible with the flight vehicle and provide unambiguous answers to simple and clear-cut questions which must be posed by the life assay program. These are formidable requirements but progress in meeting them is being made.

Several life detection systems are now under development, and they are all based on the assay of properties possessed by microorganisms or their metabolic products. These properties are:

Morphology (microscopy under visible and U.V. light)

Growth (changes in mass and turbidity)

Metabolism (changes in substrate, product)

Molecular structure (macromolecules, abundance ratios of atoms, optical rotatory dispersion, I.R. and microwave spectrometry, gas chromatography)

Devices in an advanced stage of development include:

The "Wolf Trap", a concept of Professor Wolf Vishniak (University of Rochester, New York), consists of perhaps six culture media into which dust inocula are introduced. Growth is then monitored by a photometer for increase in turbidity, and by electrodes to measure changes in pH.

"Gulliver", devised by Professor N. Horowitz (California Institute of Technology) and Dr. G. Levin (Resources Research, Inc., Washington, D.C.), relies on the detection of radioactive gases produced by the microbial metabolism of labelled substrates. C^{14} -labelled carbon dioxide is evolved as

a result of a number of different metabolic reactions by a wide variety of microorganisms incubated in a basic test medium devised for "Gulliver". This medium contains a combination of C^{14}-Formate and C^{14}-Glucose which supports the evolution of detectable levels of CO_2 by representative bacteria, both aerobes and anaerobes, streptomycetes, fungi and algae within periods ranging from a few minutes to several hours.

Time will not permit an analysis of these and other devices now being considered for automated life detection, but before proceeding with a detailed examination of our own conception, one other approach deserves particular emphasis:

It is perhaps not an exaggeration to state that molecular spatial assymetry is one of the fundamental and characteristic properties of matter associated with life. Unfortunately, measurement of dispersion and of other such optical properties does not now hold much promise for the detection of this inherent assymetry. What is needed here is a method of physical or chemical amplification of this property. An illustration of such amplification would be the initiation of selective crystallization of a large quantity of an enantiomorph by a very small seed crystal of the same material.

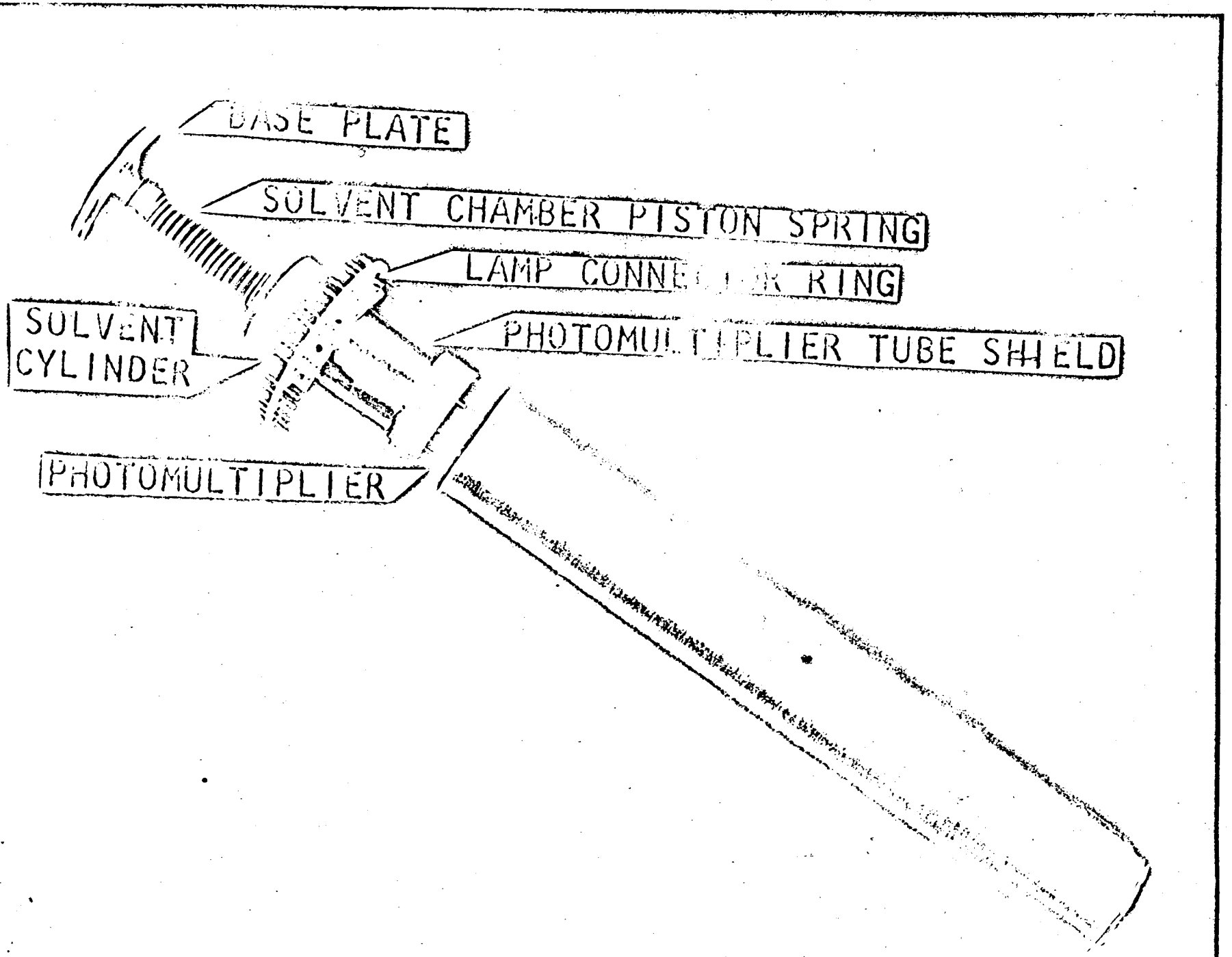
No single experiment as presently envisaged is capable of providing the necessary and sufficient condition for definitive evidence of the existence of extraterrestrial life. It was in an attempt to meet the multiple experiment requirement that "Multivator" was conceived. Multivator is a small automatic device which is designed to retrieve samples of surface dust and inject aliquots of it into fifteen modules. Each module is a small reaction chamber in which the necessary reagents are stored. Water is then introduced into all modules simultaneously and the reactions allowed to take place for a suitable period of time. Experiments and their controls are so designed as to be translated into a photometric assay, the light of which is collected by a photomultiplier. These signals are then processed and telemetered back to Earth. A large number of photometric measurements can be visualized: They include absorption at selected wave-lengths, fluorescence, phosphorescence, scattering, polarization, scintillation. Other assay methods which may also be suitable include conductimetry, pH measurements, and signals from a variety of solid-state counters. The

design of all these experiments is compatible with the limited telemetry rate of about one bit per second imposed by the power-distance ratio. The detailed discussion of "Multivator" which follows will be divided into two parts. First, the device and its operation, and second, the biological assays under development for use with "Multivator" will be analyzed.

"Multivator" - The device and its operation.

"Multivator" has undergone a number of modifications. The early model involving a rotating cell system, designed by the Jet Propulsion Laboratory in collaboration with members of our laboratory at Stanford University, has had to be abandoned because of greatly lowered weight and power limitations. Subsequently, Mr. Lee Hundley of our laboratory,and the Jet Propulsion Laboratory, independently of each other, but in mutual consultation, developed new designs to meet these restrictions. Mr. Hundley's design represented a sharp departure from the original conception. It will now be discussed before we report on a third design, now in preparation.

The basic assembly is shown in figure 1. This unit has a diameter of 2 1/2 inches, a length of 10 inches and weighs approximately one pound. The heart of the device is shown in figure 2. Fifteen reaction chambers are visible on the periphery of the disk. Twelve of these chambers are connected to the manifold base of the dust aerosol loading tube by distributing channels. The other three chambers are dust-free controls. Note that the channels enter the reaction chambers tangentially in order to impart a cyclonic movement to the entering dust and later, to the water solvent. Dust is collected in the reaction chambers by an inert viscous material such as silicone, and the residual gas is exhausted via ports located on the underside of the reaction chambers disk. This system is then closed by a


BASE PLATE
SOLVENT CHAMBER PISTON SPRING
LAMP CONNECTOR RING
SOLVENT CYLINDER
PHOTOMULTIPLIER TUBE SHIELD
PHOTOMULTIPLIER

SOLVENT LOADING PORTS
AEROSOL LOADING TUBE
AEROSOL DISTRIBUTION CHANNELS
REACTION CHAMBER
BLANK CHAMBER

Fig 2

shut-off valve which seals the manifold base of the aerosol tube, and by a slight rotation of the teflon solvent cylinder, as shown in figure 3. This slight rotation uncovers the solvent loading ports, leading to all fifteen reaction chambers, and simultaneously releases the spring loaded piston which delivers solvent to all chambers. When the piston bottoms, it shuts off and isolates every reaction chamber. The appropriate reactions are then allowed to take place for suitable intervals of time. Photometric assay of fluorescence is then carried out, in the current conception, by sequentially activating small lamps located at the lamp ports (figure 3) and electrically attached to the lamp connector ring (figure 1). The response from each chamber can then be measured by the photomultiplier and processed for telemetry. Activating and response optical filters are used to reduce the background illumination. The process of sequential photometric reading can be done as many times as necessary and feasible to detect changes as a function of time. Three experiments, with their appropriate controls and variables, can be programmed in the instrument.

Fig. 3.

A third modification of "Multivator", dubbed Mark II, is currently under development at Stanford University by Professor John E. Arnold and Mr. Alan R. Pitkanen of the Mechanical Engineering Department, in collaboration with our Exobiology laboratory. In this modified design, seals are replaced by bellows and controlled explosive charges (Squib charges) carry out mechanical operations after the samples have been collected by the reaction chambers. These important changes have been designed to improve reliability and decrease power requirements. The overall size, shape and weight of Mark II will be about the same as the earlier model. Mark II is shown diagrammatically in figure 4. After ejection from the soft-landed capsule, "Multivator" is designed to orient itself in a vertical position by means of adjustable stabilizing tripod legs. "Multivator" remains attached to the soft-landed capsule through an electrical umbilical cord.

On a signal, the collection and aerosol-generating systems are activated and dust from the surface is blown into the chambers as shown in figure 5. The reaction chambers configuration remains unchanged from the earlier model, thus maintaining the very high efficiency of dust collection achieved before (96%). A subsequent signal fires the squib charge inside the valving section, causing expansion of the large ductile (hexagonal-cut) diaphragm. This effectively seals the aerosol passages. Note also the bellows seal at the upper section of the diagram (figure 5). Another squib charge is now fired into the upper chamber located between these bellows. The solvent stored below that chamber, now shown in figure 6 (upper diagram), is injected into the peripheral reaction chambers following the piercing of the retaining diaphragm (lower diagram). This solvent-holding mechanism

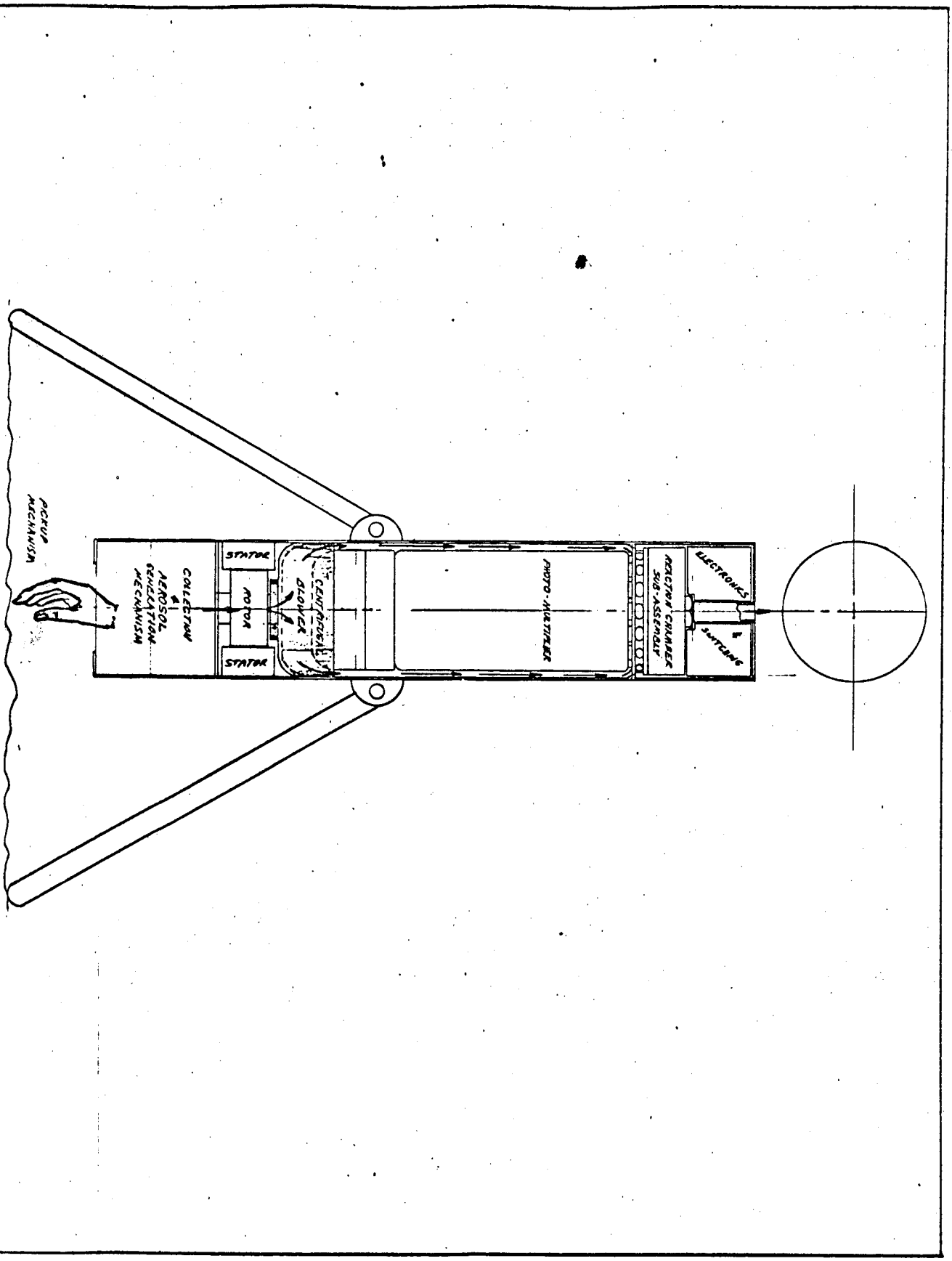

PICKUP MECHANISM
COLLECTION & AEROSOL REVOLATION MECHANISM
STATOR
ROTOR
STATOR
CENTRIFUGAL BLOWER
PHOTO-MULTIPLIER
REACTION CHAMBER SUB-ASSEMBLY
ELECTRONICS
SUNFLAME

Fig. 4

NOTES -

1. SYSTEM SHOWN OPEN TO AEROSOL FLOW STAGES 1-4 (ARROWS INDICATE PATH OF AEROSOL THRU ONE CHAMBER)

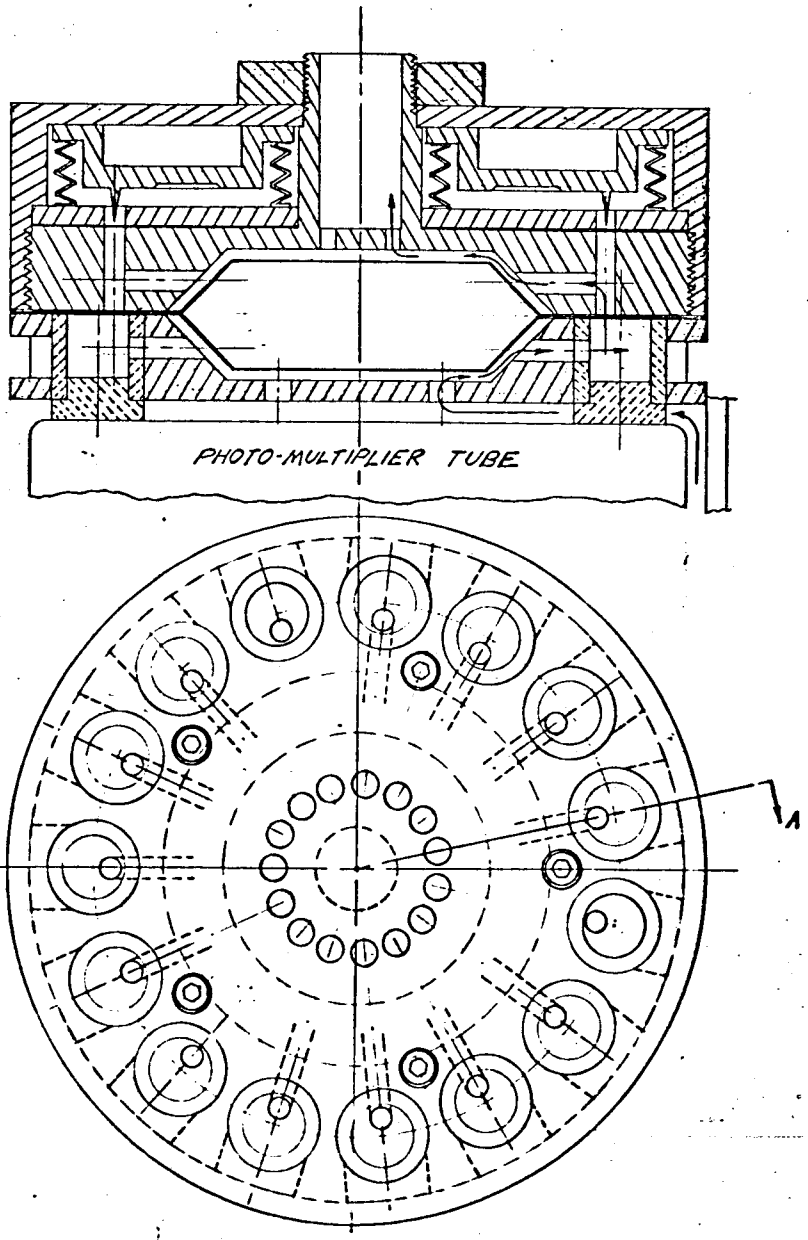


Fig. 5

NOTES -

STAGE # 5
AEROSOL PASSAGES SEALED

STAGES # 6 - 8
AEROSOL PASSAGES SEALED - SOLVENT INJECTED

Fig 6

is inserted into the device as a self-contained, independent assembly. Sealing of the fifteen injection ports is accomplished when the solvent assembly piston reaches bottom.

Programmed biological reactions and measurements are then allowed to take place in the manner described for the previous model. The data is then telemetered from the soft-landed capsule to which "Multivator" is attached. All assemblies shown here include a photomultiplier, with activation achieved through the sequential firing of gas-discharge lamps. A potentially superior method of activation, now under active development in our laboratory, would involve the use of a flying-spot scanner. An exhaustive report of specifications and performance of Mark II is expected to be made in due course.

Instrumentation of greater sophistication which is to become more appropriate for later missions is being developed by Mr. Harrison Horn of our laboratory. This includes, in particular, the use of video images and microspectrophotometry for life detection.

We now turn to the biological assay systems envisaged for "Multivator".

Biological Assays for use with "Multivator".

The choice of experiments to be carried out with "Multivator" is a difficult one to make. But certain guide lines have been established by Professor Joshua Lederberg and by Dr. Elliott Levinthal for this project. They have concluded that the experimental limitations preclude direct compositional analysis of the 100 mg soil dust samples collected by the reaction chambers. The reasonably expected abundance of living organisms in these soil dust samples (say 1,000 to 100,000 bacteria) would contain a few micrograms of DNA or protein. The detection of such minute amounts

of specified materials would be a formidable problem with all resources of an earthbound laboratory and would still leave many questions of definitive identification unanswered. At this early stage of development, therefore, we are concentrating on functional tests such as assays for enzymatic activity. Such tests, which involve admittedly riskier guesses, are capable of relatively high sensitivity and would thus significantly increase the chances of detecting life if our premises turn out to be correct.

"Multivator" could be adapted to measure one of a number of parameters associated with life functions. It is possible, for example, to synthesize artificial substrates which become fluorescent upon hydrolysis. The functional group for such a reaction may be chosen to match a particular enzymatic activity. Among these hydrolytic enzymes are the deaminases, glycosidases, sulfatases, peptidases and phosphatases. The phosphatases have received a great deal of attention in our laboratory and will be discussed in greater detail later.

Dialysis membranes are impermeable to macromolecules. If such biological macromolecules are synthesized with Carbon 14 and are subsequently broken down by enzymatic activity (depolymerases), the products permeate across the membrane and their radioactivity can then be observed by either a scintillation counter or some other detector.

Membranes can also be used for the selective diffusion of gases such as CO_2, CO or CH_4 from C^{14} labelled substrates. The evolution of such metabolic products can then be measured by an appropriate beta-detector. Both of these approaches are actively being pursued in our laboratory. Mr. Jerry Lundstrom, of our laboratory, is now making a detailed study of membrane properties and his results thus far are very promising.

A number of other functional assays are also under consideration but, for one reason or another, do not appear promising at the moment. These include the important metabolic sequences involved in photosynthesis, utilization of inorganic substrates, coupling reactions with DPN/DPNH, catalase and hydrogenase activity.

We shall now return to and report on the progress made in the development of a sensitive assay for phosphatase activity.

Phosphatase activity has been given particular attention for the following reasons:

1. It is widespread among terrestrial organisms.
2. It catalyses a wide range of reactions with moderate specificity.
3. It is involved with the unique role of phosphorus in metabolism and energy transfer, which may very well be a universal characteristic of carbon-based aqueous living systems.
4. It is capable of being detected with relatively high sensitivity.

The occurrence of phosphatase activity in terrestrial soils has been the object of a systematic study carried out in our laboratory by Dr. Larry Hochstein. The assay technique used was the fluorescent product formation which will be discussed shortly. Dr. Hochstein screened a wide variety of soils from major climatic and geological regions, including samples from the Mojave Desert sands and Death Valley salt flats. These soils were obtained from a variety of sources, including the Soil Survey Laboratory of the University of California, Berkeley. Some uncertainty exists about the effect of soil handling after collection and no data is available on this point. All samples were air-dried prior to their assay. Without a single exception, all of the fifty-nine soil samples tested were

found to possess measurable phosphatase activity. Of these, fifty-one (or 86%) exhibited phosphatase activity when assayed at pH7.6, while 95% showed activity at pH 5.6. No correlation was found between the apparent pH of the soil, and the nature of its phosphatase activity. All of these activities could be destroyed by heating, or inhibited by certain chemicals, such as mercuric chloride. In the case of mercuric chloride inhibition, a reversal could be obtained by adding a sulfide. These observations represent strong evidence for the biological nature of this phosphatase activity in soils, and for its ubiquity.

The source of these activities, however, is a different matter. Most soil bacteria are tightly bound to soil particles and no reliable viable count by a direct or indirect method could be made. Little is known about the synergistic relationship and nutritional requirements of these organisms and a wide and deep area of investigation is open in this field. We are unable to determine at this juncture whether the phosphatase activity of soils represents only a current bacterial population, or whether it includes a substantial storage and preservation of active proteins from previous generations of organisms as well. There is good evidence to suggest that clay montmorillonites are able to obsorb, stabilize and protect proteins. (McLaren, A.D., Peterson, G.H., and Barshad, I. Soil Science 22: 239 (1958)).

The reference soil, collected in the vicinity of the Stanford University Medical Center, has been used over a period of eighteen months. During that time the phosphatase activity of the sample, stored in a closed jar at room temperature, remained constant after a small initial decrease. Thus, it is not unreasonable to hope that extraterrestrial surfaces might also be able to preserve biological activity representing some of the past history of the soil.

The synthetic substrate used for these assays was alpha-naphthyl phosphate, which is commercially available. Alpha-naphthyl phosphate does not fluoresce, but alkaline solutions of alpha-naphthol exhibit a marked fluorescence peak at 460 mu, when activated with light at 336 mu. Because of the short activating wave length, there often was a considerable fluorescence background due to soil components. Quenching and stability problems in addition to marked physical adsorption of alpha-nephthol by alkaline soils in particular were overcome by appropriate controls in the laboratory but would become considerably more serious in extraterrestrial investigations. In an attempt to improve the signal-to-noise ratio for these assays, Dr. Hochstein obtained, through the courtesy of the Syntex Institute, samples of methoxyl fluorescein phosphate and fluorescein diphosphate. These substances were exhaustively purified by paper chromatography prior to use. In the phosphate ester form, both of these substrates exhibited low fluorescence, but their hydrolysis products were strongly fluorescent at 520 mu, when excited at 480 mu. Dr. Hochstein found that the relative stability of the three substrates compared as follows:

	pH 6 (Tris buffer)*		pH 8	
	4°	24°	4°	24°
Alpha-naphthyl phosphate	.001	.007	Not detectable	.003
Methoxyl fluorescein phosphate	.08	.25	.08	.33
Fluorescein diphosphate	.001	.014	.010	.025

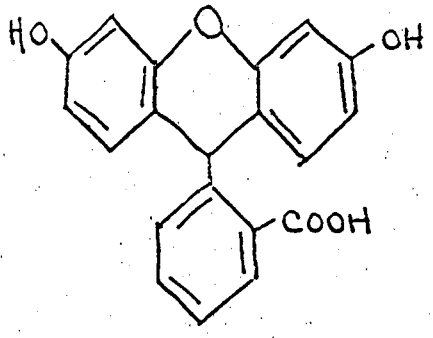
*Data obtained by fluorescence measurements, expressed in per cent hydrolysis of substrate per hour.

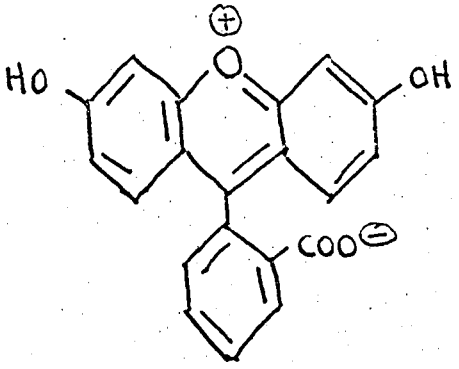
It can be seen that alpha-naphthyl phosphate is the most stable of the three, and that methoxyl fluorescein is very unstable under the assay con-

ditions used. The background fluorescence produced by these substrates corresponds to a hydrolysis rate produced in an hour, by the phosphatase activity of approximately 10^5, 10^6 and 10^7 E. coli bacteria respectively. The alpha naphthyl phosphate value does not include the soil absorption factor nor the frequent native soil fluorescence, which tended to significantly decrease the signal to noise ratio. Both the methoxyl fluorescein phosphate and the fluorescein diphosphate were found to be quite unstable and had substantial fluorescence as the phosphate esters, perhaps due to slow hydrolysis even in the solid form. In any case, while these substrate have a very high inherent sensitivity to phosphatase activity, the assay thresholds correspond to many more bacteria than we have any right to expect to find in 100 mgs. samples of extraterrestrial soils. A number of approaches are being examined in an effort to circumvent, if not to solve, these problems. Among these are attempts to increase fluorescence yields, to localize the substrate on a gel in order to detect local spots of intense fluorescence where soil particles have been deposited, then to activate and scan these areas with the flying-spot device mentioned earlier.

A number of other fluorescent substrates are being screened, but fluorescein still seems to be the most attractive possibility, if only one could overcome the unstability of its phosphate esters. One possibility has suggested itself and ought to offer a good chance of success. It is the following:

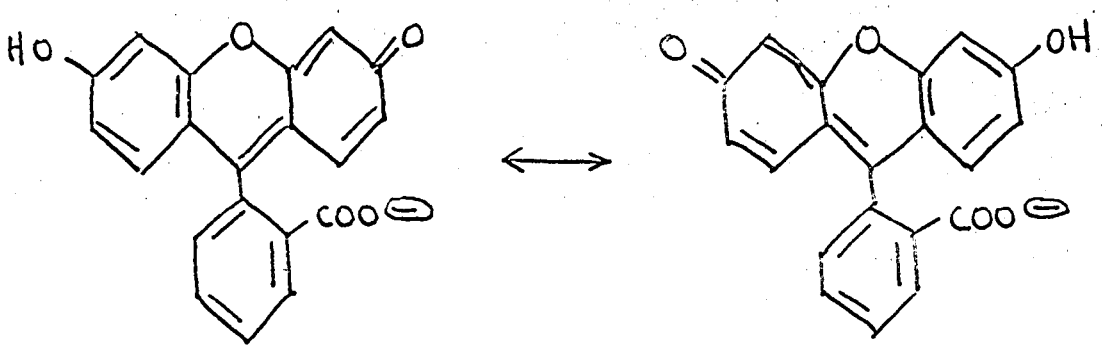
There is a great deal of confusion in the literature on the compound fluorescein. Actually two distinct compounds exist and together they represent a redox pair. They are:





Fluoresc<u>in</u> $C_{20}H_{14}O_5$ Fluoresc<u>ein</u> $C_{20}H_{12}O_5$

The oxidised substance in alkali is uranine, the structure which is the actual fluorescent hybrid:



The reduced form does not fluoresce, or fluoresces very weakly. It is slowly oxidized to fluoresc<u>ein</u> in the presence of oxygen. We also have preliminary data which suggests that light enhances the rate of oxidation. Fluoresc<u>in</u> has been used as an assay substrate for catalase, oxidases and peroxidases, in the presence of which it very rapidly oxidized to fluoresc<u>ein</u>. Fluoresc<u>in</u> seems to have another advantage. Because its hydroxyl groups are phenolic rather than quinoid tautomers, its phosphate esters ought to be considerably more stable than those of fluoresc<u>ein</u>. Since fluoresc<u>in</u> is already much less fluorescent than fluoresc<u>ein</u>, the background fluorescence should be reduced to negligible levels. Hydrolysis of fluoresc<u>in</u> diphosphate

would yield fluorescin, which could then easily be converted to the intensely fluorescent species. Or, it could be used as a double assay, for phosphatase activity as well as for oxygen, oxidase or peroxidase activity. These later activities have been universally associated with hemes and porphyrins and, according to Professor Melvin Calvin's view, they may be early components of a developing life system. I had hoped at this meeting to either refute or confirm our hopes based on this approach. But, as is usually the case, it takes considerably more time and effort in the laboratory, to translate a paper concept into a working reality. This comment applies as well to the whole effort which is being generated in the quest for extraterrestrial life.

But it is most certainly worth the try!